NO:125 and thus allows for the specific discovery of the function of this specific (beta-dystrobrevin/dystrobrevin B) gene within the broader context of mammalian physiology.

REMARKS

Claims 1-7 are presently pending. Claim 1 has been amended to address the Examiner's concerns regarding allegedly inoperative species. Support for the amended claims language can be found throughout the specification but more particularly at Page 13, at or around the header of Section 6. Accordingly, the amendments are not deemed to constitute new matter.

No prior art rejections are presently of record.

I. The Present Claims Are Patentable And The Rejections of Record Should Be Withdrawn.

a) Rejections Under 35 U.S.C. § 101

The Examiner's rejections of Claims 1-7 under 35 U.S.C. section 101 is respectfully traversed. The Examiner has apparently adopted the position that the claimed invention lacks patentable utility due to its not being supported by either a specific and/or substantial utility or a well established utility.

As discussed above, the human genome has been sequenced and, as expected, the analysis has verified that only a minor fraction of the genome is actually predicted to encode exon sequence that can be spliced, polyadenylated, and used to produce corresponding proteins. From a pharmaceutical development perspective, the critical question remains which one of these proteins present meaningful opportunities for the development of pharmaceutical products?

The Examiner is respectfully requested to consider that the specifically described novel ES cell clones are each specifically identified by corresponding exon sequences (presented in the Sequence Listing) that provide *a unique (and hence, highly specific) resource* for mapping that portion of the murine genome that encodes the described exon sequence and, by proxy, that portion of the human genome that encodes the human ortholog of the described sequences. The Examiner is requested to consider that public and private efforts have spent several billion dollars to obtain human genomic sequence data (and that corporate partners have committed to spending millions of dollars for early access to human genomic sequence), one can state that such genomic sequence

data, in part or in whole, have a demonstrated, substantial, and specific utility fully within that contemplated by 35 U.S.C. section 101 (see also the issues of "Nature" (2001, *409*:745-964) and "Science" (2001, *291*:1304-1351 that were both dedicated Human genomic sequence data). The practical implementation of the present invention adds value to human genomic data by assigning critical functional annotation to the human sequence data. It is thus axiomatic that an invention that adds value to an asset having *demonstrated and substantial economic and scientific utility* must also have substantial utility. As such, it should be clear that even in the broader context of the application as a whole (as opposed to the specifically claimed ES cell line), the presently described invention has a demonstrated substantial utility.

The Examiner is also invited to consider that the presently claimed mouse ES cell clones provide a *specific* resource for discovering the *in vivo* function of a *specific* human ortholog. In this instance, the described ES cell line mutates the murine ortholog of a human gene encoding dystrobrevin B. When the ES cells having a mutation in the gene corresponding to SEQ ID NO:125 were used to produce homozygous mutant animals, male animals homozygous for the mutated allele displayed a substantially reduced percentage of total body fat. The overt phenotype that was identified using the claimed invention clearly indicates that this gene represents one of the relatively small percentage of genes that encode proteins that present an overt commercial opportunities for pharmaceutical development– in this case, as a target for therapeutic intervention in the important fields of obesity and metabolism. This is precisely the type of demonstrated pharmaceutical utility contemplated by U.S. patent law and even satisfies the substantially more onerous U.S. Patent office utility guidelines. Given that there can be no question that the "final product"of the claimed invention has a substantial pharmaceutical utility, there can be no question that the presently described murine ES cell clone also have a substantial, credible, and well established utility. Those skilled in the art would clearly understand that the described cells having an engineered mutation in the gene encoding SEQ ID NO:125 (the dystrobrevin B gene) are particularly well, if not uniquely, suited to determining the *in vivo* function of the dystrobrevin B gene. Contrary to the Examiner's assertions, those skilled in the art would clearly not believe that "any cell comprising any nucleic acid" is equally well suited to identifying the *in vivo* function of the dystrobrevin b gene. Accordingly, the Examiner is respectfully requested to withdraw the rejection of Claims 1-7 under 35 U.S.C. section 101.

Finally, Applicants request that the Examiner consider the broader scientific acceptance of the inherent value of knockout mice to discovering the function of genomic sequence information. As evidence of such, the Applicants' respectfully direct the Examiner's attention to the recent announcement of the winners of the 2001 Lasker awards. Reproduced below for the Examiner's convenience are the comments by Lasker award presenter Ira Herskowitz:

> Albert Lasker Award for Basic Medical Research, 2001
> Comments at the Awards ceremony
> Presented by Ira Herskowitz
>
> "The release of the human genome sequence in draft form makes this a landmark year in the history of biology. Now we know that we have 30,000 or so genes (or is it 50,000?). We are now faced with several important questions, which include:
>
> First, what are the functions of these genes and the proteins that they code for? And, second, how can we use this information to improve human health?
>
> Until the ability to knock out genes in the mouse was developed, determining the function of human genes seemed largely out of reach, tantalizingly so. For example, we might know of a human protein that is found only in certain cells of the brain and suspect what it might do, but how can we find out? Or, we might know of a gene in the fruitfly that is necessary for its development and see that humans have a very similar gene. Does it perform a similar function in humans? A powerful way to link a gene to function is to study the behavior of a mutant that lacks that gene and then see what the mutant can and cannot do. It's somewhat like disabling an automobile by removing one part and then inferring the function of the part that was removed. But we can't knock out genes in a human, so how can such mutants be produced?
>
> The mighty mouse has come to the rescue. Its genes are typically 95% identical in sequence to ours, and we share the vast majority of our genes with the mouse. Despite the obvious differences between human and mouse in morphology and in some physiological processes, these differences are greatly outweighed by our similarities: they have kidneys and brains like ours; they have an immune system and develop a lot like humans; and they get diseases such as cancer and others that affect their cardiovascular and nervous systems like us. In some respects, mice are "pocket-sized humans". The bottom line is that the mouse provides the opportunity, dreamed about for decades, to make the link between a mammalian gene and its function. How is this done?
>
> Building on more than one hundred years of genetic and embryological studies of the mouse, **Mario Capecchi, Martin Evans,** and **Oliver Smithies** have created a magic wand by which it is possible to modify any mouse gene with exquisite precision -- to completely delete it or to produce a specifically altered form of the gene.
>
> The same technology also makes it possible to go the other direction - instead of knocking

out a mouse gene, it's possible to restore function to a gene that is defective.

Let's now look at the process by which a mouse knockout is constructed.

A key piece of starting material is a mouse gene that's already been cloned: it might be a mouse gene corresponding to a human gene or a mouse gene corresponding to a fruitfly or nematode gene. The goal is to construct a mouse that lacks this gene. The second key piece of starting material is a special mouse cell line where the gene is going to be knocked out.

There are three steps for constructing a mouse knockout. In the first, a cloned gene is manipulated in a test tube to delete all or part of the gene. This is routine molecular biology. In step two, the mutated DNA is introduced into special mouse cells, where the mutated DNA replaces a normal gene copy in the chromosome. The crucial aspect of this process is that the mutant gene has to find the related sequences in the chromosome, so-called homologous DNA sequences, and then undergo recombination to switch places with the good gene. The ability of the introduced DNA to find the homologous DNA sequences is called "gene targeting". There was no evidence for gene targeting in animal cells growing in culture and great doubt about whether this could be done. This is where Capecchi and Smithies made their most important contributions. In the third step, the cells with the targeted, inactivated gene are grown into a mouse that has this inactivated gene. It was Martin Evans who isolated the cell lines that made this possible and showed that genetic changes introduced into these cells in culture could be transmitted through the germ line and into mutant, progeny mice.

Let's now look at our awardees.

Verona, Italy has given us not only Romeo and Juliet, but Mario Capecchi. His early days as a child included living in orphanages and on the street in war-torn Italy from 4-9 years of age, then growing up in a nurturing Quaker environment in Pennsylvania. I refer interested people to articles that are available on the Internet. Capecchi did his graduate work at Harvard with Jim Watson and was enormously productive, making textbook discoveries on molecular mechanisms underlying protein synthesis. This was a golden age of molecular biology. Mario learned his lessons well, and when he established his own laboratory at the University of Utah in 1973, he sought to bring molecular genetics to animal cells growing in culture and learn how to manipulate the genes of these cells. This led him to undertake a series of studies beginning in 1977 that demonstrated gene targeting in animal cells and culminated in the construction of one of the first knockout mice in 1989. His first indications of homologous recombination in animal cells were published in 1982 and fueled a series of logical and remarkable studies that provide the standard methods for knocking out mouse genes.

Oliver Smithies was trained as a biochemist, but throughout his scientific career, homologous recombination kept on cropping up, and he came to think about how it could be used to fix defective genes. Smithies was born in Halifax, England and raised in the United Kingdom. After studying at Oxford University, he came to the University of Wisconsin for postdoctoral studies in 1951 and was on the faculty there for 28 years, from

1960-1988. He is presently at the University of North Carolina at Chapel Hill, and may well have flown here in his own little plane to attend this luncheon. After important early contributions springing from his development of a method for fractionating proteins, he became intrigued by the structure and evolution of mammalian genes, which meant that he became involved in cloning these genes.

In the early 1980s, Smithies began to wonder whether homologous recombination - gene targeting - could be carried out experimentally to correct a defective gene, for example, a mutant globin gene. For this type of genetic correction to occur, exogenously introduced DNA would have to target to the homologous chromosomal DNA sequence and recombine with it. But was this possible? No one had demonstrated gene targeting in animal cells.

In 1985 Smithies and colleagues demonstrated that they could introduce a DNA segment containing part of the globin gene into cells and then find cells in which this DNA segment had targeted to the chromsomal globin gene. This was a tour-de-force of sophisticated molecular genetics. His strategy was completely different from that used by Capecchi and though laborious, the demonstration of targeting was unequivocal.

These studies from the Capecchi and Smithies laboratories provided one of the essential ingredients for constructing gene knockouts in mice, the ability to target genes in cultured animal cells. The crucial next step was to take mouse cell lines modified in this manner and produce mice from them.

The history of mammalian embryology is intellectually rich and filled with great practical applications. It was nurtured by the agricultural industry among others and involved important work with rabbits and mice. The UK can lay claim to many important contributions in this area, and thus Martin Evans is part of a distinguished tradition. Evans was born in the UK and graduated from Cambridge in 1963. He then went to University College London, where he studied vertebrate development using frogs. After working with a certain type of cancer cell line that could differentiate in cell culture and be used to generate whole mice, Evans set out to isolate normal cells from an early mouse embryo that would have similar properties. Work from Richard Gardner argued for the existence of such cells, but culturing them had been elusive. In 1981, Martin Evans and Matt Kaufman and, independently, Gail Martin, in the U.S. were successful in isolating such cells, which have become known as embryonic stem cells, "ES cells". Evans then carried out an important series of experiments with his students Allan Bradley and Elizabeth Robertson that demonstrated that these ES cells could contribute to the mouse germ line. They further showed that genetically manipulated ES cells could transfer their genetic changes to progeny mice. The importance of ES cells was immediately recognized by Capecchi and Smithies, who learned how to grow ES cells and demonstrated that they could carry out targeted genetic alterations with them.

The first knockout mice constructed by gene targeting were published in 1989, and the rest is history. More than 4,000 different knockout mice have been constructed in the last dozen years, and many more are in the works! To keep on top of this fast-moving field, I suggest

looking at the Jackson Laboratory's website, where you can find columns called "It's a Knockout!" and "KO of the Month".

The ability to modify the genetic make-up of a mouse by design provides a wealth of information on the function of the gene that is knocked out. Every aspect of mammalian physiology is being penetratingly analyzed by this technique. Particularly notable are the discoveries made on how the immune system functions, which have enormous implications for human health. Knockout mice made it possible to demonstrate unequivocally the molecular basis for prion diseases such as mad-cow disease. Knockout technology is also used to create mice that have versions of human diseases such as cystic fibrosis, muscular dystrophy, atherosclerosis, and many others. These mice make it possible to follow the course of a disease and provide an opportunity to identify and test drugs to ameliorate or cure these diseases.

The ability to precisely tailor mouse genes has completely revolutionized the practice of biomedical science for the last decade and is likely to become even more important in the decades to come. We are certain to reap an enormous bounty of information from knockout mice and reap great benefits for the improvement of human health."

In view of the above remarks, there should be no doubt that the mutated ES cell clones of the present invention have a well established and credible utility. For a technical point of reference, the technology used to generate the presently described mutated clones has, in just a few years, produced and identified many fold more identified and characterized ES cells clones than have cumulatively been produced to date *by the world-wide scientific community* using the technologies that were the subject of the 2001 Lasker award referenced above.

In the event that the Examiner may still have some lingering doubts, Applicants invite the Examiner to further consider the guidance of the National Institute of Health which issued a request for applications entitled TOOLS FOR INSERTIONAL MUTAGENESIS IN THE MOUSE on January 25, 2001 (RFA-DA-01-011 which stated in part:

"This RFA solicits proposals for development of tools and techniques for the establishment of random and targeted sequence-tagged insertion libraries of embryonic stem (ES) cells that can be used to generate mutant mice in which the expression of the tagged gene could be controlled temporally and spatially. The development of such a resource for wide distribution to the scientific community would make it possible to scan the sequence database for any gene of interest and order the corresponding targeted ES cell line. Ideally, the insertional mutagenesis system developed would permit a wide range of genetic analyses and manipulations, including enhancer-trapping, conditional knockouts, conditional expression or overexpression, etc. It also would permit the larger community of investigators to utilize genomic resources efficiently. This effort complements ongoing

National Institutes of Health (NIH) efforts to create and characterize induced point mutations in mice using ethylnitrosourea (ENU) and provides a functional genomics tool to translate the information from the Mouse Genome Sequencing Project. Further information about NIH initiatives on mouse genomics and genetics resources is available at http://www.nih.gov/science/mouse."

Related to the above quote, the Examiner is respectfully invited to visit the website www.baygenomics.ucsf.edu which describes a publicly funded gene trapping effort that further supports Applicants' position that the presently described invention has a well-established utility. As a point of technical comparison, the present application alone describes over 2,000 different ES cell clones that were generated using a technology that produced more identified ES cell clones in a month than the publicly funded BayGenomics effort has produced in a given year. In brief, that branch of the U.S. government that is specifically tasked with sponsoring technologies having high biomedical utility, has already financially "validated" the utility of a related, albeit technically inferior, gene trap technology by providing many millions of dollars of funding for such efforts. In view of the direct governmental validation of biomedical utility of gene trapped mouse ES cell clones, one cannot credibly assert that the presently described ES cell clones somehow lack a well-established utility.

In view of the overwhelming evidence of the substantial, credible, specific, and well-established utility of the presently claimed invention, the Applicants' respectfully request that the Examiner withdraw the pending rejection of Claims 1-7 under 35 U.S.C. section 101.

b) Rejections under 35 U.S.C. Section 112

The Examiner has also rejected Claims 1-7 under 35 U.S.C. section 112, first paragraph because those skilled in the art would allegedly not know how to use an invention lacking a bona fide utility. Given the fact that the Applicants have used the general and well established methods described in the specification to demonstrate that the "final product" of the claimed invention indeed has a pharmaceutical utility, the Examiner cannot credibly argue that those skilled in the art would not know how to use these well established and general techniques to "use" the claimed invention. As such, the Examiner is respectfully requested to withdraw this aspect of the rejection under 35

U.S.C. section 112, first paragraph.

The Examiner has also rejected Claims 1-7 under 35 U.S.C. section 112, first paragraph over the claims being directed to "murine" ES cells. Applicants have amended Claims 3 and 7, to recite "mouse" and thus the rejection of Claims 3 and 7 over this aspect of 35 U.S.C. section 112, first paragraph is deemed to have been avoided by amendment.

The Examiner has rejected Claims 1-7 under 35 U.S.C. section 112, first paragraph for allegedly lacking written description for the claimed invention. The Examiner's rejection is respectfully traversed. The Examiner has correctly stated that the written description requirement generally requires that the specification convey to those skilled in the art that the applicant is effectively in possession of the claimed invention. What the Examiner has apparently failed to recognize is that the described method of preparing the claimed ES cell clones requires the actual production and isolation of the described ES cell clones prior to the generation of any sequence data. Those skilled in the art would clearly understand that the sequence data reported in SEQ ID NO:125 represents exon sequence that clearly identifies the gene that has been mutated in the described ES cell line (in this case the beta-dystrobrevin gene as identified in Figure 2, GENBANK accession no. AJ003007. In brief, those skilled in the art would understand that *actual possession of ES cell clones* ***is a prerequisite*** *of obtaining the exon sequence data reported in SEQ ID NO:125.* As such, those skilled in the art would have **no question** that the Applicants are in actual possession of the described ES cell clones. To the extent that the Examiner still clings to the untenable position that the gene encoding SEQ ID NO:125 may not exist in nature, the Applicants submit that the clear phenotype manifested by mice produced from the described ES cell clones also knocks out any question of whether SEQ ID NO:125 is indeed encoded by a gene whose sequence exists in nature. In view of the above considerations (and the amendment of Claim 8 to limit the claims to murine ES cells), the Examiner is respectfully requested to withdraw the rejection of Claims 1-7 under 35 U.S.C. section 112, first paragraph for lack adequate written description.

Having said such, Applicants further invite Examiner to consider that such issues of written description can routinely be dispensed with by the deposit of the described ES cell line. Although unnecessary given the teaching in the specification, Applicants will offer such a biological deposit if it remains the last impediment to issuance of the pending claims.

The Examiner has also rejected Claim 7 under 35 U.S.C. section 112, second paragraph as

allegedly indefinite because it is unclear that there is "more than one gene comprising the polynucleotide sequence * * * of SEQ ID NO:125". The Examiner's rejection is respectfully traversed in part and avoided in part (by amendment). The Applicants have amended Claim 7 to recite mouse ES cells. Thus, those skilled in the art would realize that the mutation would be in a mouse gene encoding SEQ ID NO:125 in so far the rejection relates to this Claim. Moreover, those skilled in the art would also recognize that murine ES cells are typically diploid and thus contain (with the exception of the X and Y chromosomes) at least two copies of each gene. The described mutation can occur in either of the two loci and be passed on to "offspring" cells or animals (after the mutated ES cells are used to generate mice capable of germ line transmission of the described mutation– although it remains remotely possible that both of the diploid loci can be mutated). In view of the amendment and technical considerations, the Examiner is respectfully requested to withdraw the rejection of Claims 1-7 under 35 U.S.C. section 112, second paragraph.

II. CONCLUSION

In view of the foregoing amendments and remarks, the Applicants believe that the application is in good and proper condition for allowance. Early notification to that effect is earnestly solicited.

If the Examiner feels that a telephone call would expedite the consideration of the application, the Examiner is invited to call the undersigned attorney at (281) 863-3333. The Commissioner is authorized to charge any underpayment or credit any overpayment to Deposit Account No. 50-0892 for any matter in connection with this response, including fees for any extension of time, which may be required.

Respectfully submitted,

June 30, 2003
Date

Lance K. Ishimoto Reg. No. 41,866

LEXICON GENETICS INCORPORATED
(281) 863-3333

Exhibit A

Marked Up Version of Amended Claims–U.S. Patent Application Ser. No. 09/680,959

1. (Amended) A genetically engineered mammalian cell that has been mutated by a process comprising the insertion of a recombinantly manipulated polynucleotide sequence into a gene in said genetically engineered mammalian cell wherein said gene is identifiable as corresponding to SEQ ID NO:125.

2. The genetically engineered mammalian cell of Claim 1, wherein said cell is murine.

3. (Amended) A cell according to Claim 2, wherein said cell is a mouse [an] embryonic stem cell.

4. The genetically engineered mammalian cell of Claim 1, wherein said polynucleotide sequence is present on a viral vector.

5. A cell according to Claim 4, wherein said viral vector is a retroviral vector.

6. A cell according to Claim 4, wherein said viral vector additionally comprises regions of targeting DNA that facilitate gene targeting by homologous recombination.

7. (Amended) An isolated mouse [murine] embryonic stem cell line comprising an engineered retroviral gene trap vector in at least one gene comprising a polynucleotide sequence disclosed in SEQ ID NO:125.